INTRODUCTION
Insufficient recovery of repaired lacerated skeletal muscles is commonly encountered [1,2]. As early as three months after a muscle repair, the segment distal to the laceration undergoes progressive muscle atrophy with fibrosis developing at the site of laceration [1,3]. In relation to the muscle length, the more proximal the site of the laceration the poorer the recovery [1,2]. If the site of the laceration is distal to the motor point of the muscle, it is likely that the main intramuscular nerve is also cut, and this additional defect would influence the recovery of the skeletal muscle [4,5,6]. It remains uncertain whether the muscle atrophy in the distal segment arises from the poor recovery of a disrupted intramuscular (IM) nerve or a defect in the muscle fibers per se. We hypothesized that the integrity and recovery of the cut intramuscular nerve in the lacerated skeletal muscle contributes to the healing of the skeletal muscle as a whole.

METHODS
The medial gastrocnemius (MG) in adult NZ White rabbits was used in this study (N=25) according to the guidelines laid by the Ethics Committee of the Animal Holding Unit, National University of Singapore. The left MG were lacerated completely at the proximal quarter of the muscle belly, just distal to the motor point of the branch that arises from the tibial nerve. Three different repair protocols were assessed to investigate in a lacerated muscle, the influence of the main IM nerve on muscle recovery after repair. Experimental Groups: Nerve Repair Group, NR (n=10): the main IM nerve branch at the site of the laceration was approximated by microanastomoses using 11-0 nylon sutures, under the operating microscope before the muscle was repaired; Non Nerve Repair Group, nonNR (n=10): the muscle was repaired without repairing the IM nerve; and the Nerve Preserved Group as a positive control, CP (n=5): during the simulation of the muscle laceration, with a sharp dissection, care was taken to preserve intact the IM nerve that innervated the distal segment. All lacerated muscles were immediately repaired by epimysial suturing. The non-operated contralateral MG in each animal served as the normal paired control, CS. At the assessment time-point, the MG muscle was removed and snap frozen in isopentane, cooled with liquid nitrogen. Transverse sections of the segments at the site of the laceration, as well as distal to laceration site were made for histochemistry and immunohistochemistry (IHC) staining. The nonNR and NR muscles were assessed at the 5th and 7th month postoperatively, while the positive control, CP muscles were evaluated only at 7-months. The morphology of the IM nerve in the MG was investigated with double staining of acetylcholinesterase (AchE) and HIC for neurofilament or neonatal myosin heavy chain (MHCn). Myofiber regeneration was identified by IHC for MHCn and desmin, and interstitial fibrosis was also examined by modified Masson’s trichrome and IHC for vimentin. Average cross-sectional area (CSA) of the total and regenerating myofibers and total area of scarring tissue were measured by morphometric analysis.

RESULTS
There was a marked reduction in scar tissue area across the laceration site for the NR and CP muscles but not for the nonNR muscles. There was a return of the CSA of total myofibers in segments distal to the lacerated site, in NR and CP muscles up to 7 months after repair (Fig. 1). Muscle segments distal to the laceration site in the nonNR muscles had a greater number of MHCn-positive regenerating myofibers than the NR and CP muscles even at 7-month, which was confirmed with immunostaining for vimentin and desmin. This demonstrated an active state of muscle regeneration in the nonNR muscles. On the contrary, muscle regeneration was rarely seen in NR and CP muscles. There was no significant difference in the number regenerating myofibers and the size of the scar tissue between NR and CP muscles.

Proximal IM-nerve stump with few inconsecutive nerve sprouting in the expansive scar were observed concurrently with denervation in the distal segments of the nonNR muscles. While abundant nerve fibers across the repaired site existed simultaneously with ordered reinnervation in distal segments in NR and CP muscles.

DISCUSSION
The results strongly suggest that an improved recovery of the IM nerves with repair of main IM nerve branch by microanastomosis, at the laceration site, promotes the healing of the lacerated skeletal muscle. This study offers a rationale for the repairing the concomitantly cut IM nerve branch at the lacerated site, in addition to epimysial suturing the muscle cut ends, in the treatment of lacerated skeletal muscle. It also suggests that denervation may in part account for the scar formation at the site of the laceration, and muscle atrophy in lacerated muscles. However, the cellular and molecular mechanisms at the earlier time points after repair still requires further investigation to affirm these hypotheses.

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REFERENCES

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